DOI 10.1007/s00702-005-0295-3
J Neural Transm (2005) 112: 1433–1445

Journal of Neural Transmission
Printed in Austria

Increased cell–cell adhesion, a novel effect of R-(−)-deprenyl

V. Jenei[1], **K. Zor**[1], **K. Magyar**[2], and **J. Jakus**[1]

[1] Institute of Biomolecular Chemistry, Chemical Research Center, Hungarian Academy of Sciences, and
[2] Department of Pharmacodynamics and Neurochemical Research Unit, Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary

Received August 13, 2004; accepted February 12, 2005
Published online March 23, 2005;

Summary. The neuroprotective effect of the antiparkinsonian monoamine oxidase (MAO)-B inhibitor, R-(−)-deprenyl has been under investigation for years. Cytoskeleton, a main component of cell adhesion, is involved in the development of R-(−)-deprenyl-responsive diseases, the effect of the drug on cell adhesion, however, is not known. We examined the effect of R-(−)-deprenyl on cell–cell adhesion of neuronal and non-neuronal cells. R-(−)-deprenyl treatment resulted in a cell type- and concentration-dependent increase in cell–cell adhesion of PC12 and NIH3T3 cells at concentrations lower than those required for MAO-B inhibition, while S-(+)-deprenyl was not effective. This acitvity of R-(−)-deprenyl was not prevented by the cytochrome P-450 inhibitor, SKF525A, while deprenyl-N-oxide, a newly described metabolite, also induced an increase in cell–cell adhesion. The effect of R-(−)-deprenyl was not reversible during a 24-hour recovery period. In summary, we described a new, MAO-B independent effect of R-(−)-deprenyl on cell–cell adhesion which can contribute to its neuroprotective function.

Keywords: R-(−)-deprenyl, cell adhesion, cytoskeleton.

Introduction

R-(−)-deprenyl (also known as Selegiline), which is used in the treatment of Parkinson's disease (PD) and has beneficial effects in Alzheimer's disease (AD), is not just a relatively selective inhibitor of monoamine oxidase-B (MAO-B) (Knoll et al., 1965; Knoll and Magyar, 1972). Its MAO-B-independent antiapoptotic, antitumoral and antioxidant effects were also found to contribute to its beneficial effect in the treatment of neurodegenerative diseases and some other disorders outside the central nervous system (Thyaga Rajan et al., 1995; Thomas et al., 2002; for review Magyar and Szende, 2004). There are several hypotheses trying to explain the neuroprotective effect of

R-(−)-deprenyl by its antioxidant activity (Carrillo et al., 1991) or interaction with antiapoptotic signaling pathways (Tatton et al., 1994, 1996), nevertheless, the exact mechanism of its effect is still unclear.

There is an increasing number of indirect evidences pointing towards the importance of cell adhesion in neurodegenerative disorders like AD and PD, where R-(−)-deprenyl is used. Cell-matrix and cell–cell adhesion has an important regulatory function in several cellular processes such as cell proliferation and survival by activating signaling cascades *via* integrins and cadherins. This way, adhesion can affect the survival of neurons in neurodegenerative disorders. Cell–cell adhesion is an important process during neurogenesis as well (Schlett et al., 2000; Hamada-Kanazawa et al., 2004; Laplante et al., 2004), which has recently been suggested to be involved in the pathogenesis of PD and AD (Zhao et al., 2003; Höglinger et al., 2004; Jin et al., 2004). Formation of cell–cell contacts is an essential step in synaptogenesis and structural plasticity (the reorganization of synaptic connections) (Garner et al., 2002), which are targets for protective treatments to slow AD progression and preserve cognitive and functional abilities (for review see Coleman, 2004). The role of the cytoskeleton, a central element of cell adhesion, has also been implicated in the pathogenesis of those diseases where the beneficial effect of R-(−)-deprenyl has been reported, such as neurodegeneration in PD and AD, or tumor progression. The main cytoskeletal abnormality in PD is the development of Lewy bodies in the perikarya and Lewy neurites during neuronal processes (Braak and Braak, 2000). α-Synuclein, a ubiquitous protein, is a major component of Lewy bodies, which has a role in neuroplasticity, neuroprotection and neurotoxicity (Spillantini et al., 1998). Very recently, it has been shown that the effect of α-synuclein, which under normal conditions regulates the activity of dopamine transporter (DAT) by reducing the levels of DAT localized at the plasma membrane, can be reversed to a neurotoxic action by impairment of cell adhesion under pathophysiological conditions like PD (Wersinger et al., 2003). Several proteins, which are known regulators of cell adhesion, such as presenilin-1 (Schwarzman et al., 1999), focal adhesion kinase (Grace and Busciglio, 2003), neural cell adhesion molecule (NCAM; Mikkonen et al., 1999) or intercellular cell adhesion molecule-1 (Lee et al., 2000), and β-amyloid precursor protein (Postuma et al., 2000) were found to be involved in the pathogenesis of AD. Tumor growth was also shown to be inhibited by R-(−)-deprenyl (Thyaga Rajan et al., 1995, 1999, 2000). The involvement of cell adhesion and the cytoskeleton in the regulation of cell proliferation is a well-established fact. The disruption of stable cell–cell junctions may lead to uncontrolled cell migration and proliferation during tumor development.

Although the putative role of the cytoskeleton and cell adhesion in disorders, where R-(−)-deprenyl was found to be effective, has been shown, there are no data available on the effective role of the drug in this process. The above mentioned observations prompted us to investigate whether the neuroprotective and antitumoral function of R-(−)-deprenyl can be partially attributed to an unknown effect of this drug on cell adhesion, a process directly mediated by the cytoskeleton. Consequently, the aim of our study was to examine the effect of

R-(−)-deprenyl on cell–cell adhesion using a model system including cell lines of neuronal and non-neuronal origin.

Materials and methods

Chemicals and tissue culture materials

Deprenyl-N-oxide was synthesized in the Chinoin Co. Ltd (Budapest, Hungary) and it was provided as a generous gift of the firm. Horse serum was purchased from Euroclone Ltd (Wetherby, West York, UK). 7S nerve growth factor (NGF) was obtained from Calbiochem (Budapest, Hungary). All other chemicals and tissue culture materials used were purchased from Sigma-Aldrich Co. (Budapest, Hungary).

Cell culture

NIH3T3 and NIH3T3/EGFR (NIH3T3 cells overexpressing the epidermal growth factor receptor) fibroblasts were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine serum. PC12 cells were grown in DMEM supplemented with 10% fetal bovine serum and 5% horse serum. For NGF-induced differentiation, PC12 cells seeded at low density were maintained in the above-mentioned media supplemented with 100 ng/ml NGF for 9 days. S180 mouse sarcoma cells were purchased from ECACC (Salisbury, UK) and maintained in Minimum Essential Medium of Eagle supplemented with 10% fetal bovine serum and 1% non-essential amino acids. All media contained 2 mM L-glutamine, 100 U penicillin and 0.1 mg streptomycin. All cell cultures were maintained at 37°C in a humidified atmosphere with 5% carbon dioxide.

Cell association assay

The method we used was a modified version of Sander et al. (1999). To measure cell–cell association, sub-confluent NIH3T3, NIH3T3/EGFR and S180 cells were trypsinized, while PC12 cells were removed from the plate by washing them off with medium using a pipette. Removed cells were spinned down at 2500 rpm for 5 minutes at room temperature. After discarding the supernatant, cells were resuspended in 3 ml DMEM and single-cell suspension was made by repeated pipetting. Total number of cells (Nc) was determined by counting the cells in a Neubauer improved chamber. Single cell suspensions were incubated for 2 hours (or 1 hour in case of NGF-treated PC12 cells) at 37°C in a humidified atmosphere with 5% carbon dioxide. During incubation, cells were gently mixed every 15 minutes. The extent of cell association was studied after incubation by counting the number of cell aggregates (Na) under the microscope. Cell association is represented by the ratio of Na/Nc.

Statistical analysis

After excluding the validity of parametric methods by testing the samples for normal distribution, the nonparametric Mann-Whitney U test was used to statistically evaluate the data using Statistica software'99 edition (StatSoft, Tulsa, OK, USA). Values are means ± S.E.M. in all figures.

Results

Concentration- and cell type-dependent effect of R-(−)-deprenyl on cell–cell adhesion

The effect of R-(−)-deprenyl on cell–cell adhesion was tested using the neuroectodermal-originated rat pheochromocytoma PC12 as a model of neuronal cells, and the non-neuronal NIH3T3 mouse embryo fibroblasts to represent cells outside the nervous system. To see whether neuronal differentiation affects

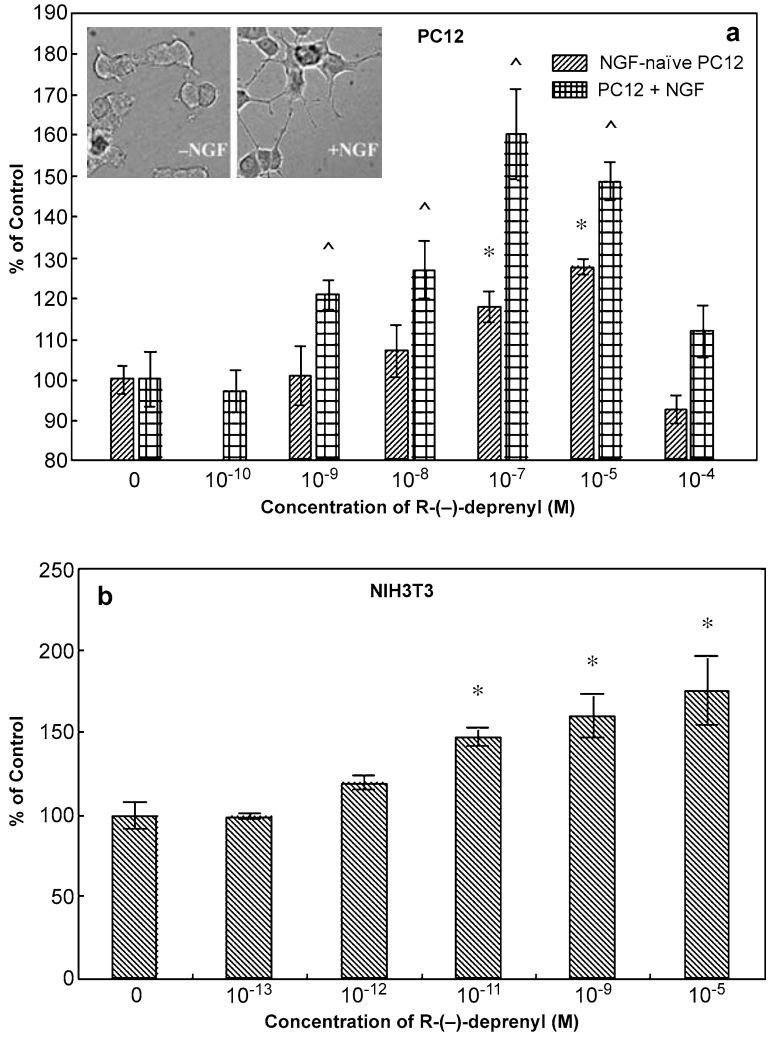

Fig. 1. Concentration-dependent effect of R-(−)-deprenyl on cell–cell adhesion. Subconfluent cells were treated with different concentration of R-(−)-deprenyl for 24 hours and cell–cell adhesion was measured as described in the Materials and methods. **a** Naïve PC12 cells, n = 8–15; *significantly different from untreated cells at $p < 0.02$; NGF-differentiated PC12 cells, n = 8–15; ^significantly different from untreated NGF-differentiated PC12 cells at $p < 0.04$; inserted image: morphological difference between naïve (−NGF) and NGF-treated (+NGF) PC12 cells; **b** NIH3T3 cells, n = 3–10; *significantly different from untreated cells at $p < 0.02$

R-(−)-deprenyl-induced cell–cell adhesion, we tested the effect of the drug on NGF-naïve and NGF-treated PC12 cells. NGF treatment induces axonal outgrowth and differentiation of PC12 cells into cell types resembling sympathetic-like neurons (see Fig. 1a, inserted images). NGF-naïve, as well as NGF-treated PC12 and NIH3T3 cells were treated with different concentrations of R-(−)-deprenyl for 24 hours at 37°C, and cell–cell association was tested as described in the Materials and methods section. The number of cell aggregates increased in a concentration-dependent manner in both cell lines after R-(−)-deprenyl treatment, however, the concentration dependency was different in the

two cell lines. A concentration of 10^{-7} M of R-(−)-deprenyl was needed to induce a significant increase of cell–cell adhesion in naïve PC12 cells, while a concentration as low as 10^{-9} M was enough to obtain the same effect in NGF-treated PC12 cells (Fig. 1a). In NIH3T3 cells a mild, statistically non-significant potentiation in cell–cell adhesion was already observed following 10^{-12} M R-(−)-deprenyl treatment, and 10^{-11} M R-(−)-deprenyl was enough to achieve significant increase in adhesion compared to the untreated cells (Fig. 1b). The maximal effect occurred after stimulation with 10^{-5} M and 10^{-7} M R-(−)-deprenyl in NGF-naïve and NGF-treated PC12 cells respectively, whereas in NIH3T3 fibroblasts doses higher than 10^{-11} M did not result in further significant increase. Regarding the maximal effect of the drug in the two cell lines, a ∼30% increase in the Na/Nc rate in NGF-naïve PC12 cells (mentioned as PC12 further on) and a ∼60% increase in NGF-treated PC12 cells could be observed compared to the control cells, whereas the same value in NIH3T3 cells was around 70%. R-(−)-deprenyl has been reported to reduce the size and number of 9,10-dimethyl-1,2-benzanthracene-induced mammary tumors (Thyaga Rajan et al., 2000). This prompted us to examine whether R-(−)-deprenyl enhanced adhesion of different tumor cell lines. As a model, we used NIH3T3/EGFR cells and a mouse sarcoma cell line, S180. After 24 h pretreatment with 10^{-5} M R-(−)-deprenyl, association assay was performed and Na/Nc ratio was determined. As presented on Fig. 2, 10^{-5} M R-(−)-deprenyl significantly increased cell–cell adhesion in all cell types, although the extent of this increase was cell type-dependent.

Effect of S-(+)-deprenyl on cell–cell adhesion

Originally, deprenyl was synthesized as a (+) and (−) racemic mixture. Besides the chemical structure, there are some major differences in the effect of the two

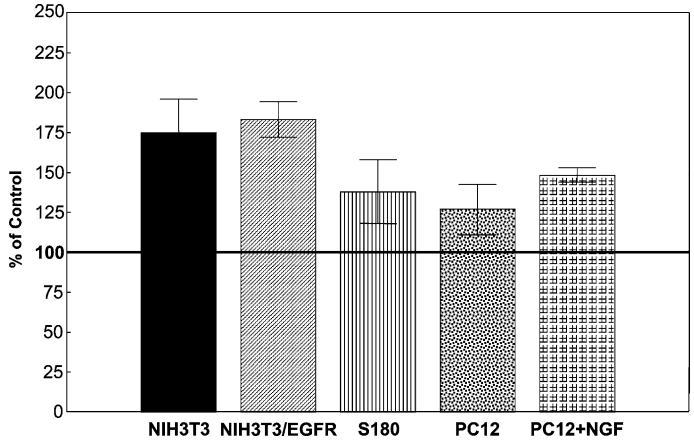

Fig. 2. Effect of 10^{-5} M R-(−)-deprenyl on cell–cell adhesion of different cell lines. Cell lines of neuronal and non-neuronal origin were treated with 10^{-5} M R-(−)-deprenyl for 24 hours and cell–cell adhesion was measured. In case of NIH3T3 cells n = 5; NIH3T3/EGFR n = 3; S180 n = 9; PC12 n = 9; PC12 + NGF n = 8. In case of all cell lines, there was a significant difference between the 10^{-5} M R-(−)-deprenyl treated cells and their respective controls at $p < 0.05$

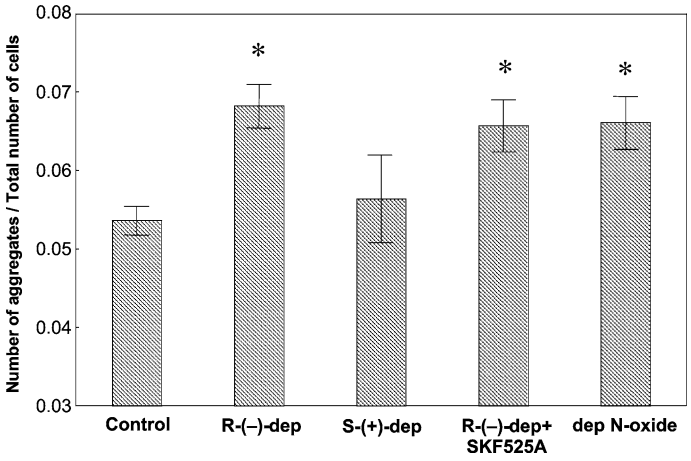

Fig. 3. Effect of S-(+)-deprenyl, the cytochrome P450 enzyme inhibitor, SKF525A and deprenyl-N-oxide on cell–cell adhesion. Subconfluent PC12 cell cultures were kept untreated (control) or treated with 10^{-5} M R-(−)-deprenyl, 10^{-5} M S-(+)-deprenyl, 10^{-5} M R-(−)-deprenyl + 10^{-5} M SKF525A or 10^{-5} M deprenyl-N-oxide for 24 hours and cell–cell adhesion was measured. *R-(−)-dep* R-(−)-deprenyl; *S-(+)-dep* S-(+)-deprenyl; *dep N-oxide* deprenyl N-oxide. n = 6–12; *significantly different from control at $p < 0.05$

enantiomers explaining why R-(−)-deprenyl became the clinically useful form of the drug. The MAO-B-inhibitory efficacy of S-(+)-deprenyl is only 1/150 that of R-(−)-deprenyl, while its metabolites have 10 times the amphetaminergic potency of R-(−)-deprenyl. The noradrenalin and dopamine uptake inhibitory potency of S-(+)-deprenyl and its metabolites is higher than that of the (−)-isomers. Based on these findings, we tested whether there is a difference between the two isomers in their cell adhesion increasing effect. We measured cell–cell adhesion of NIH3T3 (data not shown) and PC12 cells after 24 h-treatment with 10^{-5} M S-(+)-deprenyl. This treatment did not result in a significant increase of cell–cell adhesion in either of the two cell lines (Fig. 3).

Effect of metabolites on cell–cell adhesion

In vivo, R-(−)-deprenyl is metabolized by the microsomal cytochrome P-450 system resulting in the formation of three main metabolites: metamphetamin, amphetamin and desmethyl-deprenyl (Shin et al., 1997; for review Magyar et al., 2004). Recently, another metabolic pathway of the drug, the N-oxidation by flavin-containing monooxygenase (FMO) enzymes was also suggested (Katagi et al., 2001, 2002; Tábi et al., 2003), which supports the previous *in vitro* result showing that R-(−)-deprenyl is an excellent substrate for FMO (Wu and Ichikawa, 1995). In several cases it has been reported that the metabolites of R-(−)-deprenyl play a role in the pharmacological effects of the drug (Magyar et al., 1996; Szende et al., 2001). We tested the effect of SKF525A, an inhibitor of cytochrome P-450, on R-(−)-deprenyl-induced cell–cell adhesion. Furthermore, we did cell–cell association experiments using deprenyl-N-oxide, the newly established metabolite of the drug. According to our observations presented in Fig. 3, blocking the microsomal metabolism with SKF525A did not have any effect on R-(−)-deprenyl-induced cell–cell adhesion in PC12 cells,

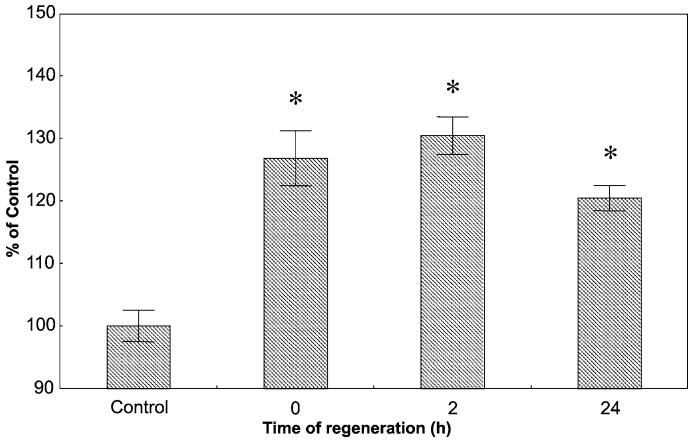

Fig. 4. Effect of 24 hours 10^{-5} M R-(−)-deprenyl treatment on cell–cell adhesion of PC12 cells after 2 or 24 hours recovery period. PC12 cells were treated with 10^{-5} M R-(−)-deprenyl. After 24 hours, medium was changed to drug-free medium for 2 or 24 hours and the number of cell–cell aggregates was measured. n = 6–9; *significantly different from control at $p < 0.05$

nor in NIH3T3 fibroblasts (data not shown). SKF525A alone did not influence cell–cell adhesion either (data not shown). SKF525A inhibits cytochrome P-450, however, there is no report showing that it affects the N-oxidation by FMO. Therefore, we can assume that deprenyl-N-oxide produced by FMO can be at least partially responsible for the effect. To test whether the increase in cell–cell adhesion upon treatment of cells with R-(−)-deprenyl can be due to this metabolite, we pretreated PC12 cells with 10^{-5} M deprenyl-N-oxide and measured cell–cell adhesion after 24 hours. Our results show that deprenyl-N-oxide significantly increased cell–cell adhesion of PC12 cells, and the extent of increase was comparable to that of 10^{-5} M R-(−)-deprenyl (Fig. 3).

Irreversible effect of R-(−)-deprenyl on cell–cell adhesion

To investigate whether R-(−)-deprenyl induces irreversible or reversible changes in the cells, we treated PC12 cells with 10^{-5} M R-(−)-deprenyl and switched to R-(−)-deprenyl-free medium after 24 hours. We let the cells recover for 2 or 24 hours and measured cell–cell adhesion as described previously. Two-hour recovery period did not induce any changes in cell–cell adhesion compared to cells that were not let to recover. Although the number of cell–cell aggregates was slightly decreased 24 hours after R-(−)-deprenyl removal, this decrease was not statistically significant compared to R-(−)-deprenyl treated cells (Fig. 4). This minor decrease in cell–cell adhesion (around 5%) was probably due to cell proliferation during the recovery period, which produced cells unaffected by R-(−)-deprenyl.

Discussion

There are no data available on the effect of R-(−)-deprenyl on cell adhesion, even though there are results suggesting the importance of the cytoskeleton and

cell adhesion in disorders where the positive effect of the drug has been shown (PD, AD and tumor formation) (Knoll, 1993; Filip and Kolibas, 1999; Thyaga Rajan et al., 1995). The goal of our study was to investigate the possible effect of this drug on cell–cell adhesion using different cell lines of neuroectodermal and non-neuronal origin. PC12 cell culture is a common model of neuronal dopamine-producing cells, like the ones affected by PD in substantia nigra. In the present study, we have demonstrated a novel effect of R-(−)-deprenyl increasing cell–cell adhesion of both NGF-naïve and neuronally differentiated PC12 cells, as well as of NIH3T3 fibroblasts, modelling cells outside the central nervous system. We observed the same effect with NIH3T3/EGFR cells, which could represent tumor cells overexpressing the EGFR, and S180, a mouse sarcoma cell line, after treatment with 10^{-5} M R-(−)-deprenyl. We can conclude that the effect of R-(−)-deprenyl is not neuronal cell-specific, but appears in non-neuronal cells, like fibroblasts or tumorigenic cells as well. This is supported by previous results showing that R-(−)-deprenyl is not only neuroprotective, but also protects the vascular endothelium from the toxic effects of amyloid-beta peptide (Thomas et al., 1998) and reduces myocyte apoptosis *in vivo* (Qin et al., 2003).

Although the precise mechanism of this unique effect of the drug needs further investigations, some conclusions can be drawn based on our results. Since PC12 cells do not express MAO-B (Youdim et al., 1986), and in NIH3T3 fibroblasts R-(−)-deprenyl induced increased cell–cell adhesion in doses that do not inhibit this enzyme (10^{-9}–10^{-13} M), we conclude that the cell–cell adhesion inducing effect of R-(−)-deprenyl is a MAO-B independent process, just like its antiapoptotic activity. Previous studies showed that very low concentrations (10^{-11} M in PC12, 10^{-13} M in melanoma) of the drug are antiapoptotic in case of cell cultures of neuronal origin like pheochromocytoma and melanoma, while at concentrations higher than 10^{-4} M the drug becomes apoptotic (Tatton et al., 2002; Szende et al., 2001). Comparing this with our observations, we can say that the results obtained in NIH3T3 cells coincide with the antiapoptotic concentration range reported for PC12 and A-2058 cells, however, both in NGF-naïve and NGF-treated PC12 cells the concentration, which increased cell–cell adhesion was somewhat higher. In our experiments, R-(−)-deprenyl failed to increase cell–cell adhesion of PC12 cells when applied at 10^{-4} M concentration, which corresponds to the previous findings of Szende and his co-workers (Szende et al., 2001). Interestingly, neuronal differentiation of PC12 cells increased the effect of R-(−)-deprenyl. As we presented on Fig. 1a, in NGF-treated PC12 cells 10^{-9} M R-(−)-deprenyl induced significant increase in cell–cell adhesion and the maximal effect of the drug was ∼30% higher than in NGF-naïve PC12 cells. This increase can be at least partially explained by the positive effect of NGF on the expression of some cell adhesion molecules like the neural cell adhesion molecule L1 as presented by others (Yip and Siu, 2001). Regarding the maximal effect of the drug in the two cell lines mentioned above, we can say that the increase in cell–cell adhesion is cell type- and concentration-dependent.

Repeating the same experiments with the MAO-B inhibitory inactive enantiomer, S-(+)-deprenyl, no significant increase of cell–cell adhesion could be

observed in either PC12 or NIH3T3 fibroblasts suggesting that structural properties of the drug must be important regarding this effect. It was shown before that S-(+)-deprenyl does not prevent apoptosis of PC12, M1, M2058 cells induced by MPTP administration or serum withdrawal (Magyar and Szende, 2004; Tatton et al., 1994).

We also examined whether the cell–cell adhesion increasing property of R-(−)-deprenyl is due to its metabolites or to the parent drug. In our experiments, treatment of cells with the microsomal drug metabolising enzyme inhibitor, SKF525A, showed no reduction of the effect of R-(−)-deprenyl, which does not agree with data on the antiapoptotic function shown to be metabolism-dependent in melanoma cells and PC12 tissue cultures (Szende et al., 2001; Tatton et al., 1997). This and the difference between the effective concentration range in apoptotic processes and cell–cell adhesion of PC12 cells suggest that the two phenomena are not based on the same mechanism. We cannot exclude, however, that the cell–cell adhesion-increasing effect of R-(−)-deprenyl can, to some extent, be attributed to its antiapoptotic effect. In our case, the results obtained with SKF525A raise two possibilities: either both the parent drug and its metabolites are equally effective, or there is no significant cytochrome P-450-dependent metabolism of the drug in our system. Abu-Raya and his colleagues showed that in PC12 cells the drug is not metabolized to any significant extent to amphetamin-like metabolites after administration of 10^{-6} M of R-(−)-deprenyl (Abu-Raya et al., 2002). Therefore, our results suggest that amphetamin-like metabolites of R-(−)-deprenyl cannot be responsible for the increase in cell–cell adhesion. In NIH3T3 cells, we cannot exclude the possibility that besides the parent drug, the formation of amphetamin-like metabolites also contributes to the effect. SKF525A is an inhibitor of cytochrome P-450 enzymes, which has not been shown to interact with FMO. Although no reference was found supporting or excluding the presence of FMO in PC12 cells, we cannot exclude the possibility that besides the parent compound, its newly identified metabolite, deprenyl-N-oxide could also be effective in inducing cell–cell adhesion. The idea was confirmed by the effect of deprenyl-N-oxide increasing cell–cell adhesion of PC12 cells.

We found that the effect of R-(−)-deprenyl on cell–cell adhesion is not reversible within a 24-hour recovery period, suggesting that the drug induces irreversible changes in the cells, which leads to increased formation of cell–cell aggregates. It has been shown by Tatton and his colleagues that R-(−)-deprenyl induces new protein synthesis (Tatton et al., 1994, 2002; Weinreb et al., 2004). It is possible that some of these proteins are cell adhesion molecules, which are responsible for the cell–cell adhesion increasing effect of the drug. Firm conclusions regarding the exact mechanism of effect, however, cannot be drawn based only on these results.

Our findings can contribute to the explanation of the beneficial effect of R-(−)-deprenyl in different disorders. The mechanism of neuroprotection by R-(−)-deprenyl, as well as the cause of neuronal cell loss in PD is still not clear. In PD and AD, accumulation of insoluble α-synuclein and, as a consequence, Lewy body formation has been shown. Alpha-synuclein has also been implicated in neuronal cell death in PD by causing oxidative stress in the dopami-

nergic neurons in the substantia nigra (Junn and Mouradian, 2002; Tabner et al., 2002). According to Wersinger and his colleagues' model, impairment of cell adhesion can lead to a disruption of the modulatory effect of α-synuclein on DAT function, which results in an increase of dopamine reuptake, oxidative stress and, as a final consequence, cell death (Wersinger et al., 2003, 2004). By increasing cell–cell adhesion of dopaminergic neurons, R-(−)-deprenyl could maintain the neuroprotective function of α-synuclein, prevent oxidative stress and neuronal cell death, however, this theory needs to be further investigated. There are a number of processes involved in neurodegenerative diseases, independent of α-synuclein, which can also be affected by cell–cell adhesion and could explain the neuroprotective function of R-(−)-deprenyl. Evidences for increased neurogenesis in the mammalian substantia nigra in a model of PD (Zhao et al., 2003) and in the hippocampus in AD (Jin et al., 2004) have been shown recently. These and other papers hypothesized that impaired or insufficient neurogenesis can be an additional explanation of neurodegenerative disorders in the late phase of PD (Zhao et al., 2003) and AD (Jin et al., 2004). The importance of cell adhesion in differentiation has been shown in NE-4C/A3 neuroectodermal progenitor cells and P19 embryonal stem cells (Schlett et al., 2000; Hamada-Kanazawa et al., 2004; Laplante et al., 2004). Furthermore, NCAM mimetic peptides have been shown to promote neuritogenesis and synaptogenesis (Kiryushko et al., 2003). In light of these new findings, we cannot exclude that R-(−)-deprenyl can develop its beneficial effects in PD and AD by affecting neurogenesis through increased adhesion and, this way, proliferation, differentiation, migration of progenitor cells and synapses-forming ability of newly formed neurons. Some studies on the effect of R-(−)-deprenyl on morphological differentiation (Koutsilieri et al., 1994; Shankaranarayana Rao et al., 1999), neuroplasticity (Zhu et al., 2000) and recovery following stroke (Sivenius et al., 2001) also suggest a role of R-(−)-deprenyl in regeneration. Establishment of cell–cell contacts is an essential step in synaptogenesis and synaptic reorganization (Garner et al., 2002). Thus, our observations could also provide an explanation of the effect of R-(−)-deprenyl on cognitive functions in AD, which have been shown in clinical trials (Falsaperla et al., 1990).

Our findings may also contribute to the explanation of the antitumoral activity of R-(−)-deprenyl. Thyaga Rajan and his colleagues found that R-(−)-deprenyl reduces the number and size of carcinogen-induced mammary tumors by enhancing splenic IL-2, IFN-γ production, NK cell activity and cathecholaminergic neuronal activities of the central and peripheral nervous system (Thyaga Rajan et al., 2000). If we assume that R-(−)-deprenyl has a similar effect on cell–cell adhesion *in vivo*, then this effect of the drug may contribute to the inhibition of tumor formation.

R-(−)-deprenyl has several effects, such as antioxidant or antiapoptotic, which can result in protection of neurons against toxic insults. In the present study, we demonstrated a completely new effect of the anti-Parkinsonian R-(−)-deprenyl on homotypic cell–cell adhesion of neuronal and non-neuronal cells, which can also contribute to its neuroprotective action by *e.g.* keeping the integrity of the cytoskeleton, inducing cell survival signals by forming cell–cell

contacts, affecting neurogenesis or dopamine uptake and reuptake through DAT. According to our knowledge, this is the first report showing this unique effect of the drug, which may have consequences in therapy, such as Parkinson's disease or tumor formation.

Acknowledgments

We would like to thank the Chinoin Co. Ltd (Budapest, Hungary) for providing us deprenyl-N-oxide and B. Lasztóczi for his help in making the images of PC12 cells.

Financial support from the QLK2-CT-2002-90436 project of the European Union for Center of Excellence in Biomolecular Chemistry is gratefully acknowledged.

References

Abu-Raya S, Tabakman R, Blaugrund E, Trembovler V, Lazarovici P (2002) Neuroprotective and neurotoxic effects of monoamine oxidase-B inhibitors and derived metabolites under ischemia in PC12 cells. Eur J Pharmacol 434: 109–116

Braak H, Braak E (2000) Pathoanatomy of Parkinson's disease. J Neurol 247: II/3–II/10

Carrillo MC, Kanai S, Nokubo M, Kitani K (1991) (−) Deprenyl induces activities of both superoxide dismutase and catalase but not of glutathione peroxidase in the striatum of young male rats. Life Sci 48: 517–521

Coleman P, Federoff H, Kurlan R (2004) A focus on the synapse for neuroprotection in Alzheimer disease and other dementias. Neurology 63: 1155–1162

Falsaperla A, Monici Preti PA, Oliani C (1990) Selegiline versus oxiracetam in patients with Alzheimer-type dementia. Clin Ther 12: 376–384

Filip V, Kolibas E (1999) Selegiline in the treatment of Alzheimer's disease: a long-term randomized placebo-controlled trial. Czech and Slovak Senile Dementia of Alzheimer Type Study Group. J Psychiatry Neurosci 24: 234–243

Garner CC, Zhai RG, Gundelfinger D, Ziv NE (2002) Molecular mechanisms of CNS synaptogenesis. Trends Neurosci 25: 243–250

Grace EA, Busciglio J (2003) Aberrant activation of focal adhesion proteins mediates fibrillar amyloid beta-induced neuronal dystrophy. J Neurosci 23: 493–502

Hamada-Kanazawa M, Ishikawa K, Nomoto K, Uozumi T, Kawai Y, Narahara M, Miyake M (2004) Sox6 overexpression causes cellular aggregation and the neuronal differentiation of P19 embryonic carcinoma cells in the absence of retinoic acid. FEBS Lett 560: 192–198

Höglinger GU, Rizk P, Muriel MP, Duyckaerts C, Oertel WH, Caille I, Hirsch EC (2004) Dopamine depletion impairs precursor cell proliferation in Parkinson disease. Nat Neurosci 7: 726–735

Jin K, Peel AL, Mao XO, Xie L, Cottrell BA, Henshall DC, Greenberg DA (2004) Increased hippocampal neurogenesis in Alzheimer's disease. Proc Natl Acad Sci USA 101: 343–347

Junn E, Mouradian MM (2002) Human alpha-synuclein over-expression increases intracellular reactive oxygen species levels and susceptibility to dopamine. Neurosci Lett 320: 146–150

Katagi M, Tatsuno M, Miki A, Nishikawa M, Nakajima K, Tsuchihashi H (2001) Simultaneous determination of selegiline N-oxide, a new indicator for selegiline administration, and other metabolites in urine by high-performance liquid chromatography-electrospray ionization mass spectrometry. J Chromatogr B Biomed Sci Appl 759: 125–133

Katagi M, Tatsuno M, Tsutsumi H, Miki A, Kamata T, Nishioka H, Nakajima K, Nishikawa M, Tsuchihashi H (2002) Urinary excretion of selegiline-N-oxide, a new indicator for selegiline administration in man. Xenobiotica 32: 823–831

Kiryushko D, Kofoed T, Skladchikova G, Holm A, Berezin V, Bock E (2003) A synthetic peptide ligand of neural cell adhesion molecule (NCAM), C3d, promotes neuritogenesis and synaptogenesis and modulates presynaptic function in primary cultures of rat hippocampal neurons. J Biol Chem 278: 12325–12334

Knoll J (1993) The pharmacological basis of the beneficial effects of (−)deprenyl (selegiline) in Parkinson's and Alzheimer's diseases. J Neural Transm [Suppl] 40: 69–91

Knoll J, Ecseri Z, Kelemen K, Nivel J, Knoll B (1965) Phenylisopropylmethyl-propinylamine (E-250), a new spectrum psychic energizer. Arch Int Pharmacodyn Ther 155: 154–164

Knoll J, Magyar K (1972) Some puzzling pharmacological effects of monoamine oxidase inhibitors. Adv Biochem Psychopharmacol 5: 393–408

Koutsilieri E, O'Callaghan JF, Chen TS, Riederer P, Rausch WD (1994) Selegiline enhances survival and neurite outgrowth of MPP(+)-treated dopaminergic neurons. Eur J Pharmacol 269: R3–R4

Laplante I, Beliveau R, Paquin J (2004) RhoA/ROCK and Cdc42 regulate cell–cell contact and N-cadherin protein level during neurodetermination of P19 embryonal stem cells. J Neurobiol 60: 289–307

Lee SJ, Drabik K, Van Wagoner NJ, Lee S, Choi C, Dong Y, Benveniste EN (2000) ICAM-1-induced expression of proinflammatory cytokines in astrocytes: involvement of extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways. J Immunol 165: 4658–4666

Magyar K, Szende B (2004) (−)-Deprenyl, a selective MAO-B inhibitor, with apoptotic and antiapoptotic properties. Neurotoxicol 25: 233–242

Magyar K, Szende B, Lengyel J, Tekes K (1996) The pharmacology of B-type selective monoamine oxidase inhibitors; milestones in (−)-deprenyl research. J Neural Transm [Suppl] 48: 29–43

Magyar K, Pálfi M, Tábi T, Kalász H, Szende B, Szökő É (2004) Pharmacological aspects of (−)-deprenyl. Curr Med Chem 11: 2017–2031

Mikkonen M, Soininen H, Tapiola T, Alafuzoff I, Miettinen R (1999) Hippocampal plasticity in Alzheimer's disease: changes in highly polysialylated NCAM immunoreactivity in the hippocampal formation. Eur J Neurosci 11: 1754–1764

Postuma RB, He W, Nunan J, Beyreuther K, Masters CL, Barrow CJ, Small DH (2000) Substrate-bound beta-amyloid peptides inhibit cell adhesion and neurite outgrowth in primary neuronal cultures. J Neurochem 74: 1122–1130

Qin F, Shite J, Mao W, Liang CS (2003) Selegiline attenuates cardiac oxidative stress and apoptosis in heart failure: association with improvement of cardiac function. Eur J Pharmacol 461: 149–158

Sander EE, Klooster JP, van Delft S, van Kammen RA, Collard JG (1999) Rac downregulates Rho activity: reciprocal balance between both GTPases determines cellular morphology and migratory behaviour. J Cell Biol 147: 1009–1021

Schlett K, Czirók A, Tárnok K, Vicsek T, Madarász E (2000) Dynamics of cell aggregation during in vitro neurogenesis by immortalized neuroectodermal progenitors. J Neurosci Res 60: 184–194

Schwarzman AL, Singh N, Tsiper M, Gregori L, Dranovsky A, Vitek MP, Glabe CG, St George-Hyslop PH, Goldgaber D (1999) Endogenous presenilin 1 redistributes to the surface of lamellipodia upon adhesion of Jurkat cells to a collagen matrix. Proc Natl Acad Sci USA 96: 7932–7937

Shankaranarayana Rao BS, Lakshmana MK, Meti BL, Raju TR (1999) Chronic (−) deprenyl administration alters dendritic morphology of layer III pyramidal neurons in the prefrontal cortex of adult Bonnett monkeys. Brain Res 821: 218–223

Shin HS (1997) Metabolism of selegiline in humans. Identification, excretion and stereochemistry of urine metabolites. Drug Metab Dispos 25: 657–662

Sivenius J, Sarasoja T, Aaltonen H, Heinonen E, Kilkku O, Reinikainen K (2001) Selegiline treatment facilitates recovery after stroke. Neurorehabil Neural Repair 15: 183–190

Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M (1998) alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. Proc Natl Acad Sci USA 95: 6469–6473

Szende B, Bökönyi J, Bocsi J, Kéri Gy, Timár F, Magyar K (2001) Anti-apoptotic and apoptotic action of (−)-deprenyl and its metabolites. J Neural Transm 108: 25–33

Tábi T, Magyar K, Szökő É (2003) Chiral characterization of deprenyl-N-oxide and other deprenyl metabolites by capillary electrophoresis using a dual cyclodextrin system in rat urine. Electrophoresis 24: 2665–2673

Tabner BJ, Turnbull S, El-Agnaf OM, Allsop D (2002) Formation of hydrogen peroxide and hydroxyl radicals from A (beta) and alpha-synuclein as a possible mechanism of cell death in Alzheimer's disease and Parkinson's disease. Free Radic Biol Med 32: 1076–1083

Tatton WG, Ju WY, Holland DP, Tai C, Kwan M (1994) (−)-Deprenyl reduces PC12 cell apoptosis by inducing new protein synthesis. J Neurochem 63: 1572–1575

Tatton WG, Wadia JS, Ju WY, Chalmers-Redman RM, Tatton NA (1996) (−)-Deprenyl reduces neuronal apoptosis and facilitates neuronal outgrowth by altering protein synthesis without inhibiting monoamine oxidase. J Neural Transm [Suppl] 48: 45–59

Tatton WG, Chalmers-Redman RME, Ju WY, Waida J, Tatton NA (1997) Apoptosis in neurodegenerative disorders: potential for therapy by modifying gene transcription. J Neural Transm [Suppl] 49: 245–268

Tatton WG, Chalmers-Redman RME, Ju WJH, Mammen M, Carlile GW, Pong AW, Tatton NA (2002) Propargylamines induce antiapoptotic new protein synthesis in serum- and nerve growth factor (NGF)-withdrawn, NGF-differentiated PC-12 cells. J Pharmacol Exp Ther 301: 753–764

Thomas T, McLendon C, Thomas G (1998) L-deprenyl: nitric oxide production and dilation of cerebral blood vessels. Neuroreport 9: 2595–2600

Thomas T, Bhavnani BR, Thomas P (2002) Inhibition of human LDL oxidation by the neuroprotective drug l-deprenyl. Neurol Res 24: 169–173

Thyaga Rajan S, Meites J, Quadri SK (1995) Deprenyl reinitiates estrous cycles, reduces serum prolactin and decreases the incidence of mammary and pituitary tumors in old acyclic rats. Endocrinology 136: 1103–1110

Thyaga Rajan S, Madden KS, Stevens SY, Felten DL (1999) Inhibition of tumor growth by L-deprenyl involves neural-immune interactions in rats with spontaneously developing mammary tumors. Anticancer Res 19: 5023–5028

Thyaga Rajan S, Madden KS, Stevens SY, Felten DL (2000) Anti-tumor effect of L-deprenyl is associated with enhanced central and peripheral neurotransmission and immune reactivity in rats with carcinogen-induced mammary tumors. J Neuroimmunol 109: 95–104

Weinreb O, Bar-Am O, Amit T, Chillag-Talmor O, Youdim MB (2004) Neuroprotection via pro-survival protein kinase C isoforms associated with Bcl-2 family members. FASEB J 18: 1471–1473

Wersinger C, Prou D, Vernier P, Sidhu A (2003) Modulation of dopamine transporter function by alpha-synuclein is altered by impairment of cell adhesion and by induction of oxidative stress. FASEB J 17: 2151–2153

Wersinger C, Vernier P, Sidhu A (2004) Trypsin disrupts the trafficking of the human dopamine transporter by alpha-synuclein and its A30P mutant. Biochemistry 43: 1242–1253

Wu R-F, Ichikawa Y (1995) Inhibition of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine metabolic activity of porcine FAD-containing monooxygenase by selective monoamine oxidase-B inhibitors. FEBS Lett 358: 145–148

Yip PM, Siu C (2001) PC12 cell utilize the homophilic binding site of L1 for cell–cell adhesion but L1-$\alpha v\beta 3$ interaction for neurite outgrowth. J Neurochem 76: 1552–1564

Youdim MB, Heldman E, Pollard HB, Fleming P, McHugh E (1986) Contrasting monoamine oxidase activity and tyramine induced catecholamine release in PC12 and chromaffin cells. Neuroscience 19: 1311–1318

Zhao M, Momma S, Delfani K, Carlen M, Cassidy RM, Johansson CB, Brismar H, Shupliakov O, Frisen J, Janson AM (2003) Evidence for neurogenesis in the adult mammalian substantia nigra. Proc Natl Acad Sci USA 100: 7925–7930

Zhu J, Hamm RJ, Reeves TM, Povlishock JT, Phillips LL (2000) Postinjury administration of L-deprenyl improves cognitive function and enhances neuroplasticity after traumatic brain injury. Exp Neurol 166: 136–152

Authors' address: V. Jenei, Institute of Biomolecular Chemistry, Chemical Research Center, Hungarian Academy of Sciences, P.O. Box 17, 1525 Budapest, Hungary, e-mail: veronika@chemres.hu